SOME EFFECTS OF ANDROGENS ON THE ADULT MALE FUNDULUS

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Introduction

Concurrent with Matthews (1938, 1939*a*, 1939*b*), studies (Burger, 1939, 1940, 1941) have been made on the factors which influence the sexual cycle of the adult male *Fundulus heteroclitus*. These authors have found that warm water accelerates spermatogenesis in sexually inactive fish and that cold water retards the appearance of spermatogenesis and delays the testicular involution that follows the breeding period. Day-length has no effect on the testicular cycle. They have further found that *Fundulus* pituitaries implanted into hypophysectomized or normal males will stimulate spermatogenesis. The present study deals with the effects of two androgens,[1] principally testosterone propionate, on the sexual cycle of the adult male *Fundulus*. The literature contains few references to the effects of androgens upon the sexual cycles of adult fish. Pertinent references will be introduced in their proper context.

Materials and Methods

In all experiments freshly captured adult males, 7.5–9.5 gm., were used. In each experiment there was the same number of control and experimental fish, and like numbers of fish of approximately the same size. The controls received the same treatment as the experimentals save that hormone was not administered to the controls. For each experiment the environment was identical for the control and experimental groups. These groups were segregated in salt-water tanks. For Experiments 1, 2, 4 the water temperatures were in the main near 13°–14° C.; for Experiment 3 the water temperature was 17°–19° C. Testes were trimmed and weighed after fixation in Bouin's fluid. The precise treatments for each experiment will be found in the *Results*.

[1] Testosterone propionate (Perandren), and testosterone were kindly supplied by the Ciba Pharmaceutical Products, Inc.

Results

The periods covered by these experiments were from the full breeding state (June and July) to complete testicular involution and the preliminary spermatogonial multiplications for a new cycle (August–November). In *Fundulus* there is no abrupt transition from the breeding to the involuted condition in the testis. The gonads do not suddenly cease to form sperm; even when the testes have involuted to almost minimal size, a few sperm continue to be formed. Instead, there is a gradual decline from July to September in the number of sperm formed. The male ducts which are almost wholly intra-testicular in *Fundulus,* also slowly involute from a condition where the ducts are long and distended with sperm to one where they have degenerated into a mass of stromal tissue in which individual ducts can no longer be recognized.

The yellow coloration of the body, characteristic of the breeding male, likewise slowly disappears. Not until late August do the males completely lose this coloration, which is most obvious on those parts of the body where there is the least melanin pigmentation (ventral surface and fins). In the fairly cold water used in Experiments 1, 2, 4, these regressive changes go more slowly than they do in the warmer water of the natural habitat.

Exp. 1. Between June 27 and August 2, twenty-eight adult males, hypophysectomized on June 23–24, each received intraperitoneally, ap-

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PLATE I

Explanation of Figures

Fig. 1. Cross-section of a testis from a breeding male at the time when hypophysectomies were performed. The peripheral zone of spermatogenetic activity (s.z.) is broad. The more central duct system (d.) is well developed. Black areas are sperm.

Fig. 2. Cross-section of a testis from a fish kept in a cold-water laboratory tank 6/23–8/8. The spermatogenetic zone is still well developed, but the duct system shows some involution. The testis is smaller than that of a breeding male. Sections from testes from fish injected with 8 mg. testosterone show the same condition as shown in this figure.

Fig. 3. Cross-section of a testis from a control hypophysectomized fish killed 8/1. The spermatogenetic zone (s.z.) is almost devoid of spermatids. The testis is very much smaller than that shown in Figure 2.

Fig. 4. Cross-section of a testis of a control hypophysectomized fish, killed 8/8. Involution is more advanced than that shown in Fig. 3.

Fig. 5. Cross-section of a testis from a hypophysectomized fish, 8/1, which received intraperitoneally 7 mg. of testosterone propionate in oil. Spermatogenesis is more in evidence than in the controls (cf. Fig. 3). The ducts are also better maintained.

Fig. 6. Cross-section of a testis from a hypophysectomized fish, 8/8, which was injected intraperitoneally with 8 mg. of testosterone propionate. The spermatogenetic zone (s.z.) is less active than on 8/1 (Fig. 5), but the duct system (d.) is still well developed.

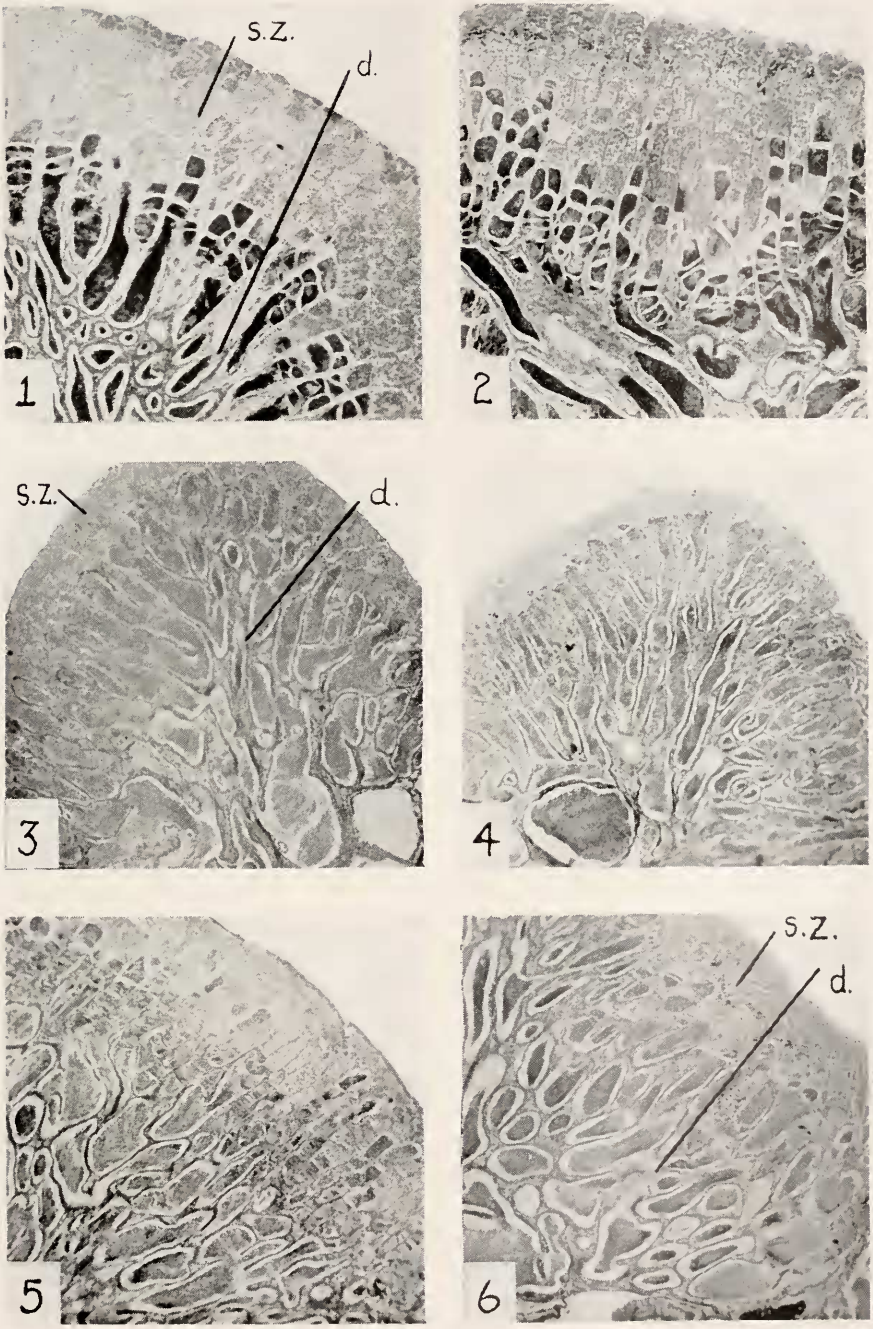

PLATE I

proximately 8 mg. of testosterone propionate dissolved in sesame oil, in equally spaced doses of 1 mg. Fish were sacrificed on August 1 and 8.

At the time of hypophysectomy, the testes were actively forming sperm (Fig. 1); the peripheral zone of spermatogenetic transformations (s.z.) is deep. The more centrally located ducts (d.) are distended and elongate. A section of the testis of a normal laboratory fish at the time of the end of the experiment (Aug. 8) is shown in Figure 2. The only difference between this testis and one from the breeding season (Fig. 1) is a slackening of spermatogenetic activity and a very slight involution of the duct system. The testicular states present in the hypophysectomized controls on August 1 and 8 are seen in Figures 3 and 4. Here spermiogenesis has almost ceased; only scattered cysts of spermatids can be found. The duct system has been markedly reduced. The testes of the hypophysectomized controls were only half as heavy as those of normal controls.

The hypophysectomized fish which were injected with 8 mg. of testosterone propionate over a 42 day period, had testes which weighed [2] about one-fifth more than those of hypophysectomized controls. Cross sections of the testes of hormonally treated fish are shown in Figures 5 and 6. It is obvious that testosterone propionate has not maintained the testes as well as they were maintained in normal fish (cf. Fig. 2). The involution is not as marked however, as in the hypophysectomized controls (cf. Figs. 3 and 4). Stages of spermatogenesis are more in evidence and the duct system is better preserved. While these differences between hormonally treated and control fish are constant, the testes of the treated fish are also near the end of their spermatogenetic activity. The spermatogenetic-stimulating effects of testosterone propionate with the methods used, are slight. The regressive changes due to hypophysectomy far outweigh any gametogenetic effect of the hormone.

The hormonally treated hypophysectomized fish maintained the full yellow breeding coloration, while most of this coloration was lost in the hypophysectomized controls. Since the nuptial mark (Parker and Brower, 1935) is lost more slowly than is the yellow coloration, a positive statement on this character cannot be made. The contact organs (Newman, 1907) were not studied.

Exp. 2. Between June 27 and August 2, twenty normal males each received intraperitoneally, approximately 8 mg. of testosterone dissolved in oil, in spaced doses of 1 mg. Animals were sacrificed August 1 and 8.

No differences in testicular states were found after a 42 day period between the control and hormonally treated fish. Figure 2 illustrates

[2] The comparisons of testicular weights must be considered only as rough comparisons.

the condition of these fishes' testes. The hormonally treated fish retained the yellow body coloration better than the controls but less well than it was retained by the hypophysectomized males treated with testosterone propionate.

Exp. 3. Between October 9 and 29, six normal males were injected intraperitoneally with approximately 6 mg. of testosterone propionate in oil in spaced doses of 1.5 mg. Animals were killed November 3.

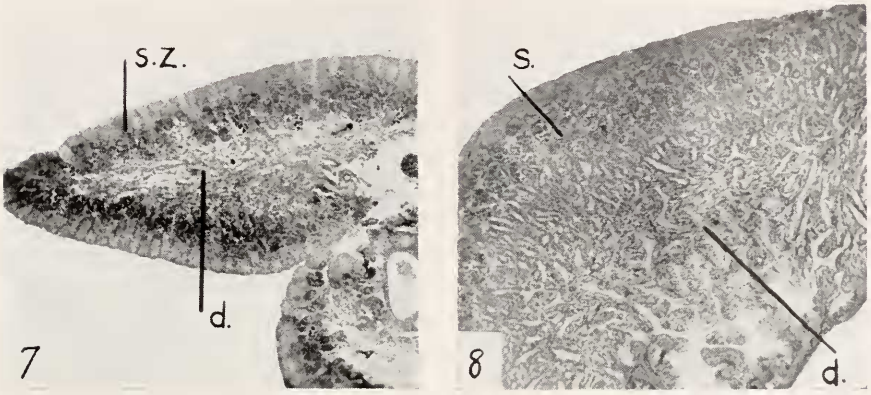

PLATE II

Explanation of Figures

Fig. 7. Cross-section from a normal control male, 11/3, after 25 days in a laboratory tank. The ducts (d.) are represented by the central stromal core, about which is the spermatogonial zone (s.z.) in the early stages of spermatogonial multiplication.

Fig. 8. Cross-section of a testis from a normal fish, 11/3, injected intraperitoneally with 6 mg. of testosterone propionate. The duct system (d.) has hypertrophied and individual ducts are visible. The spermatogenetic zone shows spermatids (s.). Essentially the same testicular state was present in fish receiving 3 mg. of testosterone propionate intramuscularly.

On October 9 and 25, six normal males each had approximately 3 mg. (2 doses of 1.5 mg.) of crystalline testosterone propionate implanted into the dorsal musculature. To effect this implantation, crystals of the hormone were tamped into the tip of a hollow glass needle. A wire plunger, snugly fitting the bore of the needle, forced the hormone into the muscles. One scale was removed from the fish. At the site of removal the needle was inserted. The scale was replaced to cover the wound. Animals were sacrificed on November 3.

A section of a normal control's testis is shown in Figure 7. Such a fish was kept 25 days in a laboratory tank. The testicular involution of the previously active gonad has been accomplished, and the prolifera-

tion of spermatogonia for a new cycle has started. Spermatids are as yet rare. The testes of the fish treated with 6 mg. of intraperitoneally injected hormone were about four times as heavy as those of the controls. The testes of the fish in which 3 mg. of testosterone propionate were implanted intramuscularly were about twice as heavy as the controls.

The histological picture was essentially the same for both groups of treated fish, with the testes from the fish injected intraperitoneally being the more advanced. Figure 8 shows a section from a testis from a fish which received intraperitoneal injections of the hormone. The most conspicuous effect of the hormone was the re-elaboration of the duct system of the gonad. Instead of being represented as in the controls by a mass of stroma (Fig. 7, d.), the individual ducts have become individually discernible. The walls of the ducts are thickened and ciliated. The duct tissue now forms the bulk of the testis and is responsible for most of the increased weight of the gonad.

The effect of the hormone on spermatogenesis was again present, but again not marked. In the treated fish there was a slight acceleration of spermatogenesis. This acceleration was not enough to present widely differing pictures between the germ cells of the controls and the hormonally treated fish. While spermatids were much more numerous in the treated fish than in the controls, numerous spermatozoa were not found. It was shown (Burger, 1941) that pituitary material could induce numerous spermatozoa in colder water and in a shorter length of time than the 25 days of this experiment.

The effect of the hormone on the yellow coloration of the body was clear. Within ten days after the first injection, yellow had developed on the body and the fins. At the end of the experiment the hormonally treated fish were brilliantly yellow. In the same period, the controls remained devoid of this color, save for one fish which showed the faintest tint. It must be remembered that the controls were slowly undergoing sexual stimulation from the warm water of the experiment.

Exp. 4. Beginning August 16, ten normal males each received intraperitoneally, approximately 9 mg. of testosterone propionate in oil in 3 mg. doses spaced at intervals of seven days. Fish were sacrificed on September 3.

On August 15, six normal males had approximately 1.5 mg. of testosterone propionate implanted into the dorsal musculature. These animals were killed on September 3.

The results of these experiments were essentially similar to those of Experiment 3. After 18 days of intraperitoneal treatment with hormone the testes were half again as heavy as those of the controls. The fish treated with intramuscular hormone had testes two-fifths heavier

than those of the controls. The hormonally treated fish were more yellow than the controls.

Discussion

In these experiments effects of testosterone propionate were observed upon the yellow coloration of the body, the intra-testicular duct system, and the germ cells. This hormone maintained the full yellow coloration of the breeding male for 42 days after hypophysectomy. In the hypophysectomized controls this coloration was largely lost. In normal sexually inactive males, testosterone propionate, injected either intraperitoneally or intramuscularly, caused a re-appearance of this yellow coloration. Controls did not become similarly colored. In normal breeding males, testosterone, milligram for milligram, was less effective than was testosterone propionate in hypophysectomized males. The results indicate that the suffusion of the body with yellow during the breeding period is normally caused in the adult male *Fundulus* by an androgenic substance. Female *Fundulus* do not show this coloration.

Within the testis the most conspicuous effect of testosterone propionate was upon the duct system. In normal sexually inactive fish the hormone produced a marked hyperplasia of the ducts. In hypophysectomized males, the hormone, with the methods used, only partially maintained the duct system. The duct system was, however, better maintained than in the controls. Testosterone with the methods used had no discernible effect on the duct system.

The effects of testosterone propionate on the germ cells are not unequivocal. In the mammal it has been shown that large doses of androgens maintain the normal condition of the testis if the injections are given before degenerative damage has occurred (Walsh, Cuyler and McCullagh, 1934; Nelson and Gallagher, 1936; Hamilton, 1936; Cutuly, McCullagh and Cutuly, 1937). In the normal adult *Lebistes,* Eversole (1939, 1941) reports somewhat contradictory results. In his 1939 paper testosterone propionate was found to have no effects in experiments of short duration and to be inhibitory to spermatogenesis in experiments of three months duration. In his 1941 paper the hormone is said to have the effects of pregneninolene, which latter substance hastens germ cell maturations without replacements by new cells and causes a hyperplasia of the testicular stroma. Eversole considers the male hormone of *Lebistes* to be more similar to pregneninolene than to testosterone propionate. In the lizard, *Sceloporus,* Forbes (1941) finds that implanted pellets of testosterone propionate stimulate spermatogenesis and produce a hypertrophy of the epididymis and vas deferens. He criticizes the

negative results of Gorbman (1939) as being the result of too short an experiment.

Our results indicate that testosterone propionate has, within the methods used, only a very slight stimulating effect on the male germ cells of *Fundulus*. This effect did not suppress the involution changes that follow hypophysectomy, although the hormone did very slightly retard this involution. In normal sexually inactive fish, this hormone did not markedly accelerate a new spermatogenesis. We have shown (Burger, 1941) that within the time limits of these experiments, pituitary material will produce copious sperm in normal and hypophysectomized *Fundulus*. It is interesting to note that the response to implanted whole pituitaries is primarily one of germ cell activation, and not one of stimulation of the ducts. With testosterone propionate, the effect seems to be the reverse, i.e., marked activation of the duct system and weak activation of the germ cells. The effect noted on the germ cells may not be a direct effect at all. It may be that by stimulating the duct system, which is intra-testicular, the *milieu* of the testis is more favorable for spermatogenesis. It must be remembered that in these experiments the germ cells of the treated fish were not in stages which differed widely from the controls.

It next must be asked whether or not the methods and the dosages employed were adequate for securing pertinent results. In comparison with other data from other workers the use of at least 1 mg. of testosterone propionate per 7.5–9.5 gm. fish per week seems to be a fairly high dosage. The use of intraperitoneal injections of the hormone in an oil vehicle may be questioned on the grounds that it does not provide for a satisfactory absorption of the hormone. Deansley and Parkes (1937) report that intraperitoneal testosterone has little effect in the mammal. Rubinstein and Kurland (1941) do not agree with this conclusion, but Greene and Burrill (1941) cast doubt on the validity of the comparisons made by Rubinstein and Kurland. Experiment 3 clearly shows that in *Fundulus* 6 mg. of intraperitoneal testosterone is somewhat more effective than 3 mg. of intramuscular hormone. The hormone in both methods of administration affected in like fashion the testis and the body coloration. Thus, it can be stated that effective amounts of testosterone propionate can be absorbed after intraperitoneal and intramuscular administration. It is clear from Experiment 2, that the amount of testosterone used was inadequate to produce testicular stimulation.

The intraperitoneal method of administration of the hormone is for small fish more convenient than are intramuscular and subcutaneous methods. In these latter two methods the repeated injection of large

doses is not practical, due to the wounds inflicted and the delicateness of the dermal epithelium. Since *Fundulus* is a very wasteful feeder with non-living food, the oral administration of the hormone would be wasteful of the hormone, and would make a reasonably accurate determination of the dosage difficult.

Summary

Adult male *Fundulus* hypophysectomized during the breeding season were injected intraperitoneally with 8 mg. of testosterone propionate during a 42 day period. The hormone maintained the yellow body coloration characteristic of a breeding male. The hormone did not maintain the testis in the breeding condition. The testicular ducts were maintained better than they were in the controls. There was slightly more spermatogenetic activity than in the controls. Like amounts of testosterone injected intraperitoneally into normal fish over a similar period had no effect on the testis and only partially maintained the yellow coloration of the body.

Normal adult male fish injected intraperitoneally or intramuscularly with testosterone propionate (see text for dosage) during testicular involution and during the early stages of spermatogonial multiplications showed a marked activation of the testicular ducts and a slight stimulation of spermatogenesis. The yellow coloration of the body developed brilliantly in the treated fish. The above experiment was performed once for an 18 day period, and once for a 25 day period.

It is concluded that the yellow coloration of the body, characteristic of the breeding male, is stimulated by the male hormone, and the elaboration of the testicular duct system is influenced by this hormone. While a slight stimulation of spermatogenesis was observed, the degree of stimulation does not warrant the conclusion that in the intact animal the male hormone has any important spermatokinetic role.

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